AMERICAN SOCIETY FOR MICROBIOLOGY | Microbiology and Molecular Biology Reviews®

REVIEW

Emerging Roles of Functional Bacterial Amyloids in Gene Regulation, Toxicity, and Immunomodulation

Nir Salinas,[a] Tatyana L. Povolotsky,[b] Meytal Landau,[a] Ilana Kolodkin-Gal[b]

[a]Department of Biology, Technion–Israel Institute of Technology, Haifa, Israel

[b]Department of Molecular Genetics, Weizmann Institute of Science, Rehovot, Israel

Nir Salinas and Tatyana L. Povolotsky contributed equally to this article. The order was determined on the basis of these two authors contributing equally to the writing of the paper but Nir Salinas also contributing a figure (Fig. 3).

SUMMARY Bacteria often reside in multicellular communities, called biofilms, held together by an extracellular matrix. In many bacteria, the major proteinaceous component of the biofilm are amyloid fibers. Amyloids are highly stable and structured protein aggregates which were known mostly to be associated with neurodegenerative diseases, such as Alzheimer's, Parkinson's, and Huntington's diseases. In recent years, microbial amyloids were identified also in other species and shown to play major roles in microbial physiology and virulence. For example, amyloid fibers assemble on the bacterial cell surface as a part of the extracellular matrix and are extremely important to the scaffolding and structural integrity of biofilms, which contribute to microbial resilience and resistance. Furthermore, microbial amyloids play fundamental nonscaffold roles that contribute to the development of biofilms underlying numerous persistent infections. Here, we review several nonscaffold roles of bacterial amyloid proteins, including bridging cells during collective migration, acting as regulators of cell fate, as toxins against other bacteria or against host immune cells, and as modulators of the hosts' immune system. These overall points on the complexity of the amyloid fold in encoding numerous activities, which offer approaches for the development of a novel repertoire of antivirulence therapeutics.

KEYWORDS amyloids, *Bacillus subtilis*, biofilms, curli, phenol-soluble modulins, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella enterica* serovar Typhimurium, TasA, extracellular matrix

INTRODUCTION

While historically thought of as unicellular organisms, in nature, bacteria form complex and differentiated multicellular communities known as biofilms. The coordinated action of the community residents improves the ability of the community to attach to hosts and protect them from environmental stresses (1, 2). The multicellular

Citation Salinas N, Povolotsky TL, Landau M, Kolodkin-Gal I. 2021. Emerging roles of functional bacterial amyloids in gene regulation, toxicity, and immunomodulation. Microbiol Mol Biol Rev 85:e00062-20. https://doi.org/10.1128/MMBR.00062-20.

Address correspondence to Meytal Landau, mlandau@technion.ac.il, or Ilana Kolodkin-Gal, Ilana.kolodkin-gal@weizmann.ac.il.

Published 25 November 2020

nature of biofilms confers unique phenotypic abilities to the residing bacteria. Therefore, biofilms, and not planktonic cells, are the bacterial entities mostly affecting their environment. One example is the enormous impact of biofilms on human health. The U.S. Centers for Disease Control and Prevention (CDC) has estimated that bacterial biofilms are responsible for 60% of chronic infections, including burn wounds, chronic ulcers of limbs associated with diabetes, periodontitis, osteomyelitis, chronic wounds and cystic fibrosis lungs (3–5). Bacterial biofilms are able to evade the host immune system and withstand treatment with antibiotics (6–8). It has been demonstrated that infection-forming biofilms are 10- to 1,000-fold less susceptible to antibiotics than their planktonic counterparts (4, 7). However, the mechanisms supporting this phenotypic resistance, as well as those driving the transition from free-living bacteria to a differentiated biofilm community, are poorly understood (4).

Biofilms are often composed of a heterogeneous community organized into a complex three-dimensional (3D) structure. The 3D structure of the biofilm was suggested to relieve metabolic stress by the utilization of channels formed below the ridges and wrinkles within the colony that may facilitate the diffusion of fluids, nutrients, and oxygen (9–12). The resulting different levels of oxygen, nutrients, and quorum-sensing molecules affect the genetic programs expressed by the cells within the biofilm and generate spatial and temporal heterogeneity (13–18).

The formation of a biofilm is a developmental process, in which various genetic programs are activated in a specific order in different subpopulations of cells, for the proper establishment of a functional structure (10, 12, 14, 19–22). This apparent coordination can be explained by the temporally distinct exposure of cell subpopulations to specific microenvironments (14).

To form a functional structure, biofilm cells produce polymers that constitute the extracellular matrix (ECM), where they bind to each other and to the surface. The ECM plays an important role in the resistance and resilience of the entire biofilm community (13, 23, 24). Although the ability to generate an ECM appears to be a common feature of multicellular bacterial communities, there is remarkable diversity in the means by which these matrices are constructed (25). The most extensively studied components of biofilm organic ECMs are carbohydrate-rich polymers (i.e., extracellular polysaccharides or exopolysaccharides [EPS]), proteins, nucleic acids (25, 26), and biogenic minerals (27, 28).

Here, we will review a major component of the proteinaceous ECM, namely, proteins which form amyloid fibrils. Known biofilm-associated amyloids include the curli-producing CsgA/B proteins produced by *Escherichia coli* and *Salmonella enterica* serovar Typhimurium (29), phenol-soluble modulins (PSMs) produced by *Staphylococcus aureus* (30), FapB/C produced by *Pseudomonas aeruginosa* (31), and TasA produced by *Bacillus subtilis* (32, 33). Importantly, bacterial amyloids can be roughly divided into intrinsic and facultative bacterial amyloids (34). Curli represent intrinsic bacterial amyloids. For these functional amyloids, the amyloid state represents the primary structural and functional state of the proteins, which are part of a biosynthetic pathway dedicated to the assembly of the functional amyloid structure (29, 34). On the other hand, TasA, belonging to a protein family with enzymatic function (33), represents facultative amyloids where protein subunits can attain a globular folded state (35), as well as a fibrous amyloid (33, 36). Bacterial amyloids that are composed of peptides, such as the *S. aureus* PSMαs ($\sim$20 residues in length), are too short to form a defined globular tertiary structure in their soluble state. Nevertheless, they can form helices and β-rich structures and mixtures of these species, in both their soluble and fibrillar states (37, 38). The dynamics between secondary structures and mixture of conformations, as well as soluble/insoluble states, even within identical sequences, challenge structure-function-fibrillation studies and prohibit a clear definition of the amyloid role in specific activities.

Functional amyloids are extremely common in bacterial biofilms and their assembly is important for the integrity of biofilms. Here, we will focus on bacterial amyloids within the ECM of microbial biofilms. While many functional amyloids in biofilms are

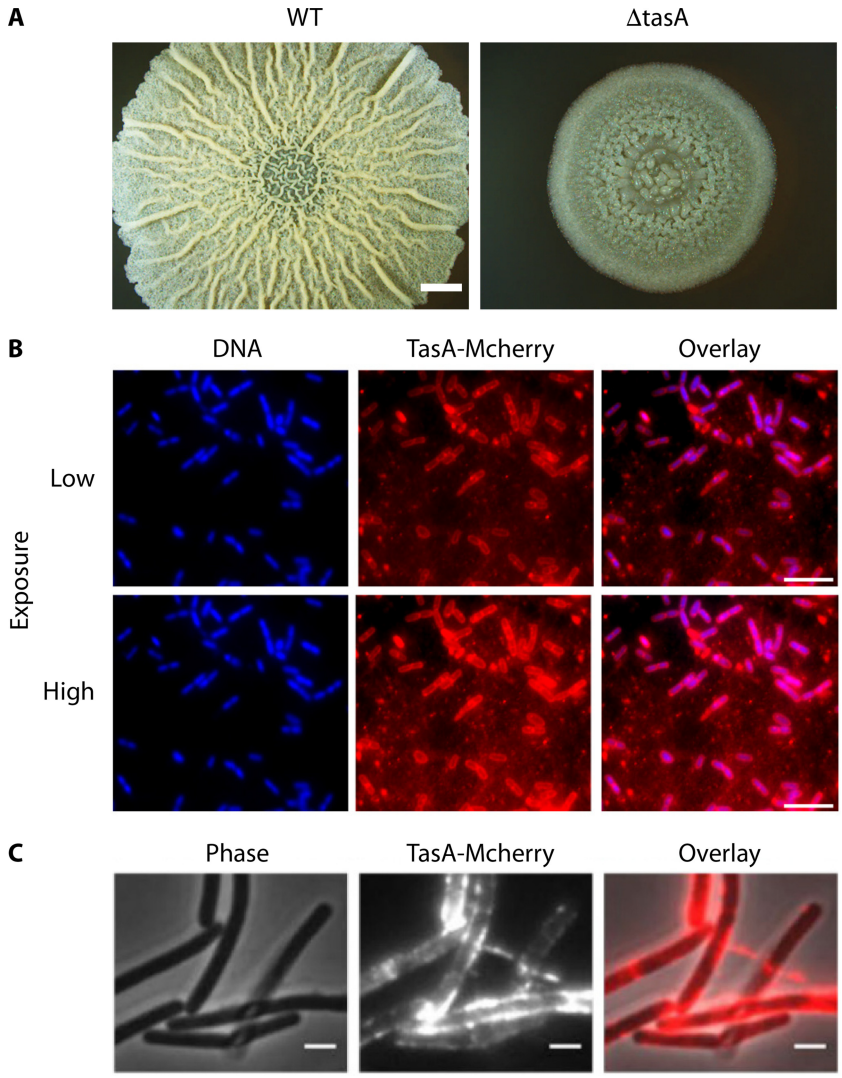

FIG 1 TasA fibrils in *B. subtilis* biofilms. (A) Biofilm colonies formed by the parental strain and a TasA mutant. Scale bar, 1 mm. (B) A functional TasA-mCherry fusion generating a network between biofilm cells. Scale bar, 2 μm. (C) Images of a fibril formed by TasA-mCherry between cells grown in a microfluidic device. (Reprinted from reference 80.) Scale bar, 2 μm.

suggested to play roles as stable and resistant scaffolds, several nonscaffold roles are emerging, including in signaling, toxicity toward competitors, and immunomodulation.

FUNCTIONAL AMYLOIDS IN COLONY STRUCTURE

Amyloids are known for their remarkable stability, ascribed to their shared cross-β structural feature, composed of tightly matted β-sheets with β-strands situated perpendicular to the fibril axis (39–50). Bacterial amyloids were suggested to share the typical cross-β structure of human amyloids, which provide ultrastability to the ECM. Thus, though the primary structure (amino acid sequence) of amyloids may not share similarities between organisms, the quaternary structure is overall similar. Amyloids are highly resistant to chemical, proteolytic, and enzymatic degradation and therefore provide a strong scaffold for the community and act almost like a shield under stress (51, 52). Many amyloids, such as curli, produced by *E. coli* and *S.* Typhimurium (29), FapC produced by *P. aeruginosa* (53) and TasA produced by *B. subtilis* (Fig. 1) (32), play a role in the formation of complex colony morphology on agar plates. Biofilms formed by amyloid-proficient strains have a morphology different from biofilms formed by

amyloid-deficient strains. Amyloid-deficient strains form flat biofilms, compared to the mature biofilms produced by amyloid-expressing strains (32, 53, 54) (Fig. 1A).

The exact role played by functional amyloids in colony morphogenesis remains to be determined. From the common contribution of amyloids to structural complexity, it was purposed that amyloid formation is a common mechanism to attain architectural complexity in biofilms. This view is compatible with recent studies and indicates a widespread and important function for the macrostructure of the biofilm in the formation of wrinkles and channels and a proper assignment of different cell types coordinating and dividing tasks within biofilms (55–57). Amyloids promote cell-cell interactions, as well as cell-to-surface adhesion, in conjunction with other components of the extracellular matrix, such as exopolysaccharides and extracellular DNA (eDNA), an additional important structural component of many bacterial biofilms (58, 59). Amyloids are known to interact with eDNA, supporting the stability of the ECM as a whole. Similarly, human amyloids also interact with DNA; for example, prion conversion of proteins into amyloid fibrils is modulated by the presence of DNA and RNA nucleic acids, and Amyloid-β, involved in Alzheimer's disease, has been shown to be structurally affected by interacting with DNA *in vitro* (60, 61). In microbes, curli and eDNA form irreversible complexes resistant to DNase I and RNase H. The eDNA is embedded within the curli fibers, which in turn protect the eDNA from degradation (62, 63). In *S. aureus*, it has been shown that the amyloid-forming secreted PSMα1 peptide also interacts with DNA. *S. aureus* strains that allow autolysis and the release of eDNA promote the polymerization of PSMα1, which further stabilizes the biofilm (64).

Amyloid fibers are considered a rigid scaffold upon which the more flexible and amorphous EPS are overlaid. When amyloid deficient mutants are complemented with either pure extracted amyloids or amyloid-producing strains, they are able to form phenotypically wild-type biofilms (65). This phenomenon of wild-type phenotype restoration by the complementation with purified protein of amyloid mutants also occurs in pellicles, biofilms that form in the water-air interface (32), and indicates that amyloids are a shared resource within the biofilm. The severe developmental defects in mutants for amyloid formation indicate that functional amyloids are an important matrix component that provides the structural "backbone" of the biofilm (Fig. 1A) (66). Therefore, the use of anti-amyloid drugs, including compounds originally aimed to treat human amyloids, emerged as a strategy to reduce the formation of pathogenic biofilms (67–69) and thereby restore their sensitivities to antimicrobials.

THE FUNCTIONAL AMYLOID TasA OF *B. SUBTILIS* AS A REGULATOR OF CELL FATE

Bacillus subtilis, a beneficial bacterium from the *Firmicutes* phylum, is highly related to emergent pathogens, among them *Bacillus cereus*, *Listeria monocytogenes* (70), prevalent food-borne pathogens, and the bioterror agent *Bacillus anthraces* (71). Undomesticated strains of *B. subtilis* are efficient colonizers of the plant host and can protect their host from fungal and microbial infections (72). The major proteinaceous component of *B. subtilis* ECM is the protein TasA, encoded by the *tapA-sipW-tasA* operon (32, 73), and colonies formed by *tasA* deletion mutants are smaller and less structurally complex than those of wild-type strains (74) (Fig. 1A). TasA forms amyloid fibers (32, 74, 75) that are attached to the cell wall and, in conjunction with other extracellular components, promote cell-cell adhesion (32, 76) (Fig. 1B and C).

The structure of the TasA protein itself is polymorphic. Diehl et al. have demonstrated that *in vitro*, TasA has two distinct structures: a globular monomeric form that is composed of two antiparallel β-sheets flanked by six helices and longer loop regions assembled into jellyroll fold, as well as a fibril form composed of mainly β-sheets in the canonical cross-β architecture (36). In contrast, Erskine et al. demonstrated that under native conditions TasA fibers are assembled from globular protein units arranged in helical repeats (35). The latest study revealed that, in its fibrillar amyloid form, TasA is composed of β-sheet and α-helical secondary structure and that this combination makes it an atypical functional amyloid (33).

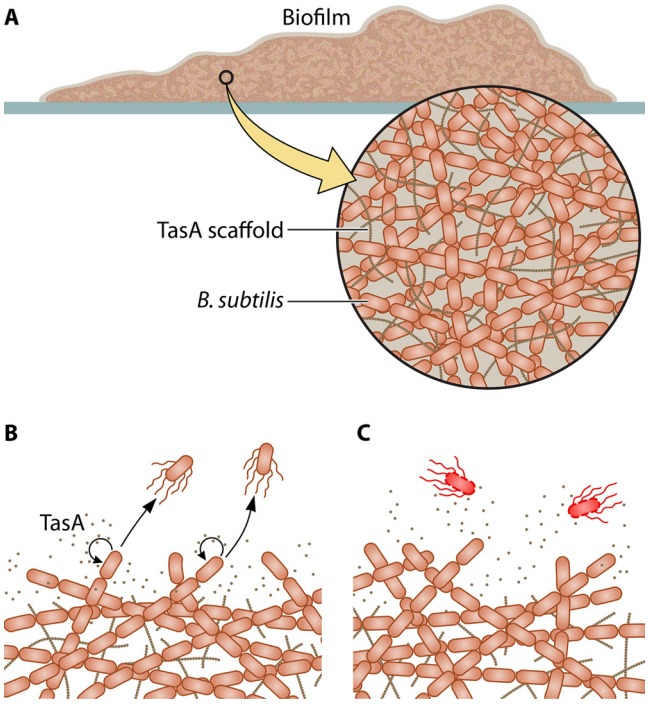

FIG 2 Scaffold and nonscaffold roles of TasA. Illustrated are the versatile functions of TasA. (A) Scaffold and adhesin roles. (B) Signaling roles (possibly exerted by the nonamyloid forms of the protein), protecting its producers from stress and inducing the formation of motile daughter cells. (C) A toxin role.

In addition to its canonical role as a scaffold promoting colony architecture, TasA has several nonscaffold functions in a biofilm. The most well-known role is as a regulator of cell fate, although many of the molecular downstream pathways for this protein remain unknown. Originally, the TasA protein was described as having antibiotic activity (77). TasA forms small aggregate intermediates that are recognized by the A11 "anti-oligomer" antibody (32), which might correspond to the toxic activity ascribed to small aggregates that are precursors to amyloid fibers (78). It is possible that TasA exists outside the cell both as fibrils that play scaffold roles and as small aggregates that defend the cells within the biofilm from potential competitors. These combined effects might lead TasA-producing cells to become resistant to antimicrobial activity (1, 77). Thus, TasA may potentially be coexpressed with its own set of resistance mechanisms, similarly to many antimicrobial compounds (72); however, the exact nature and function of these potential mechanisms remain unknown.

Collective evidence suggest that TasA can regulate microbial development, independently of its role as an ECM scaffold (Fig. 2). An analysis of the spatiotemporal gene expression profiles of a *B. subtilis* ECM mutant lacking both exopolysaccharides and TasA fibers demonstrated alterations in the number and localization of motile cells, ECM producers, and sporulating cells within the mature colony (79). Specifically, the motility-specific reporter displayed reduced expression in a TasA mutant strain. In contrast, the transcription of the ECM operons was dramatically increased in the TasA mutant (79).

TasA was recently suggested to maintain the motile cell subpopulation and to thereby induce motility. Single-cell analysis revealed that TasA is acting locally to stimulate the ECM producers to switch back to the motile state, with Δ*tasA* cells remaining much longer in the biofilm state. Within the biofilm colony, the emergence of motile cells from preexisting matrix-producing chains can allow a uniform distribution of cells, rather than the emergence of two spatially segregated subpopulations. This can promote collective migration of biofilm cells since it requires colocalization of both flagellated cells and matrix producers (80). Recent motility-focused transcriptomic

analysis of the *tasA* mutant and its signal peptidase supported that it acts as a regulator in its secreted form (80).

Similarly to many biofilm formers, in *B. subtilis*, a regulatory switch couples activation of ECM production with the repression of motility (81–84). This regulatory switch depends mainly on two master regulators that jointly control both motility and biofilm development: the homologous proteins SinR and SlrR (85). During planktonic growth, SinR represses the expression of the ECM-producing operons *epsA* to *epsO* (*epsA–O*) and *tapA-sipW-tasA*, as well as the expression of *slrR*. Once the biofilm state is induced, SinR is deactivated by SinI, resulting in activation of the ECM operons and *slrR* (86–88). In turn, SlrR binds to SinR, creating a heterodimer that represses the *fla/che* operon, which encodes key components of motility (82) and genes encoding autolysins, which are important for breaking down the cell wall so that cells can separate from one another. Thus, the same regulator, SinR, represses either the ECM operons or motility, but not both simultaneously in the same cell, and therefore the two transcriptional programs are mutually exclusive at the single-cell level (85). Interestingly, *tasA* deletion had no influence on the number of motile cells in cells lacking motility-biofilm switch master regulators and thus unable to enter the biofilm state (80). Therefore, TasA serves as an upstream signal to the motility-biofilm switch, to increase the switching from matrix production back to motility.

Consistent with the regulatory role of TasA in motility gene expression, it was found to promote microbial migration in two complementary settings. Although *B. subtilis* does not make vortices during swarming on solid media (89), it was recently shown to exhibit vortex-like motion in liquid during early stages of biofilm formation (90). TasA was found to link large localized groups of cells traveling in a common circular path (generating a "vortex") in liquid. Similarly, TasA generates bridges between cells, which promoted flagellar independent motility (sliding) on agar plates (91, 92). This suggests a dual evolutionary pressure on the structure and function of TasA: flexibility for promoting collective motility and the formation of scaffolds for the nonmigrating biofilm population.

The effect of TasA on gene expression was recently shown to be broad, prolonged, and sustainable (93). Another transcriptome study indicated that beyond motility and ECM production, *tasA* has a role in maintaining proper activation of secondary metabolite production, metabolic activities, and additional vital functions. The interaction of TasA with the bacterial membrane is proposed to play a part in its signaling roles. Membrane dynamics were suggested to be regulated by TasA, as in its absence, impaired respiration was observed, which led to an increase in the generation of reactive oxygen species (ROS) (93). The formation of ROS could account for some of the transcriptome alterations observed in the *tasA* mutant as ROS induce a full range of transcriptional and cytological alterations in microbial systems (94, 95).

TasA was also shown to be required for the expression of stress genes involved in antibiotic tolerance (80, 93). In addition to ROS formation, the secretion of TasA itself may affect gene expression. Consistently, a nonsynonymous mutation in the histidine kinase domain of the histidine kinase CssS, part of the CssRS two-component system (involved in responding to secretion stress) (96) was found to partially rescue gene expression defects in a TasA mutant strain. Because the *cssRS* deletion increased the expression of motility genes and inhibited ECM gene expression, the CssRS may sense high levels of secreted TasA. CssRS was previously demonstrated to activate several pathways involved in cell stress tolerance (80, 97–99). Since the secretion stress response is conserved in other amyloid producers, such as *E. coli* (100), it may be activated similarly to induce stress tolerance gene expression during the secretion of amyloids. Collectively, these results indicate that TasA production itself grants the producers increased endurance to stress.

Overall, the findings discussed here present a case for several arguments. First, sensing TasA may initiate a local negative-feedback loop for its own production by inducing a sharp decrease in the expression of ECM genes, acting upstream to the SinI/SinR/SlrR switch. In this way, TasA switches cells back to express motility genes, and

its secretion and sensing serve as a bet-hedging strategy to maintain both cell types in the biofilm. Since motility and biofilms are tightly connected in the microbial domain (54, 101), it remains to be explored what is the exact mechanism of sensing TasA and whether it is extendable to additional amyloids.

The gradual increase in amyloid secretion and amyloid interaction with the cell membrane can also serve to increase the resistance of microbial communities to antibiotics produced by their competitors and to stress, providing benefits to the TasA producers themselves, rather than acting as a "public good." A variant in *tasA* could restore the regulatory role, but not colony structure (93), and therefore it is unlikely that transcriptional defects of the *tasA* mutant can be solely attributed to the scaffold roles of the amyloid. Nevertheless, it remains to be determined what structural variants of the protein account for its nonscaffold functions.

STRUCTURAL POLYMORPHISM ENCODE DIFFERENT ACTIVITIES OF THE *S. AUREUS* PSM FUNCTIONAL AMYLOIDS

Highly pathogenic strains of *S. aureus*, for example, methicillin-resistant *S. aureus* (MRSA), which is responsible for more than 90% of multidrug resistance infections (102), show high expression levels of PSMs (103). Specifically, PSM peptides comprise nearly half of the secreted protein mass (104) and greatly contribute to pathogenicity (105). PSMs act as virulence determinants mainly by structuring the biofilms and by targeting the host defense system, including lysing neutrophils and other cell types, and inducing proinflammatory activity by interacting with formyl receptor 2 (FRP2) and other receptors in the immune system (37, 105–110).

The *S. aureus* PSM family is comprised of eight short peptides: PSMα1 to PSMα4, expressed from the α-*psm* operon; PSMβ1 and PSMβ2, expressed from the β-*psm* operon; and PSMγ (also known as the δ-toxin) expressed from the *hld* gene encoded within the Agr regulatory RNA gene *rnaIII* (105, 111). PSMs are α-helical amphipathic peptides and, as such, were initially identified by hot phenolic extractions from cultures of *S. epidermidis* (111, 112). Mass spectrometry studies showed that PSMs are truncated *in vivo* to generate an array of shorter derivatives, thus expanding the array of active species produced by PSMs (38, 113–117). Recent studies yielded evidence of amyloid-like fibril formation of PSMαs that play roles in mediating their virulent activities, including biofilm formation (PSMα1 and PSMα4), toxicity against host immune cells (PSMα3), and antibacterial activity (PSMα3 derivatives) (30, 37, 38, 110, 118, 119).

Biofilm formation facilitates *S. aureus* attachment and colonization of tissues or medical implants and protects the bacterium from antibiotics and immune response, leading to chronic and persistent infections (120). Analyses of the biomolecule composition of the *S. aureus* biofilm matrix revealed, among polysaccharides and eDNA (64), a proteinaceous fibrous material, consisting mostly of PSMα1 and PSMα4 in an amyloid aggregated state (30). Biophysical and structural characterization of PSMα1 and PSMα4 revealed that they self-assemble into cross-β amyloid fibrils (38). The PSMα1 and PSMα4 short segments IIKVIK and IIKIIK, respectively, which are conserved among naturally occurring truncations (113–117), were identified as the spine segments of the fibrils (38). Atomic resolution structures of these spine segments showed β-sheets that tightly mat through steric zipper interfaces, adopting the ultrastable cross-β fibril architecture typical of segments of human disease-associated amyloids (38) (Fig. 3). Subsequent studies showed the formation of cross-β steric zipper structures also in segments from CsgA, the main amyloid subunit of curli in the biofilm of *E. coli* (67). The cross-β fibrillar architecture shown for the first time in bacteria raises hypotheses regarding the evolution and physiological roles of amyloids across kingdoms of life. The involvement of the cross-β amyloid architecture in structuring the biofilms of different bacteria offers novel targets for the development of anti-biofilm compounds, which could potentially reduce the aggressiveness of infections and increase susceptibility to conventional antibiotic treatments. Accordingly, known anti-amyloid compounds were successfully repurposed as antibiofilm agents (67).

PSMα3, the most cytotoxic peptide in the PSM family, is also a key player in MRSA

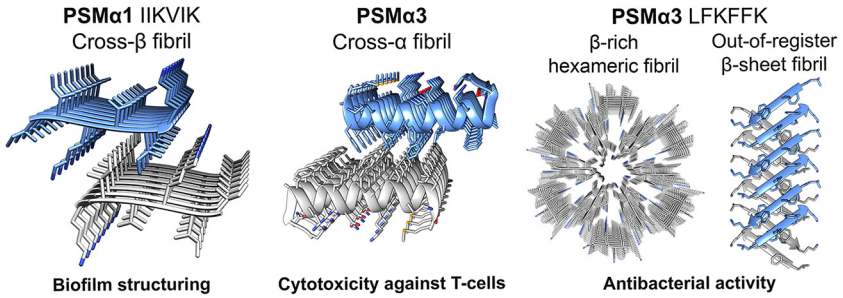

FIG 3 Structural heterogenicity of phenol-soluble modulins (PSMs). Shown are resolved crystal structures of indicated PSMs.

acute infections by utilizing a strategy to evade the host immune system. PSMα3 kills neutrophils after phagocytosis and also lyses lymphocytes, erythrocytes, and other cell types (103, 105, 107, 109, 110, 121–123). PSMα3 cytolytic activity occurs in a membrane perturbation fashion, rather than being receptor mediated. Recently, it was shown that PSMα3 self-assembles into fibrils with a novel amyloid cross-α architecture of α-helices which stack perpendicularly to the fibril axis, forming matted sheets, as did the β-sheets in the cross-β fibrils (Fig. 3) (110). The PSMα3 structure encompassed the full length of the amyloid's sequence, a feature that has never before been achieved by crystallography. The secondary structure polymorphism observed for the PSMα3 cross-α fibrils compared to human cross-β amyloids was striking and indicative of structurally encoded functional specificity, particularly when considering that homologous family members, PSMα1 and PSMα4, form the canonical cross-β amyloid fibrils (38).

PSMα3 cross-α fibrillation was suggested to serve as a critical determinant of toxicity against human T cells (37, 110, 118). In a mutagenesis study, it was shown that a few nonfibrillating mutants, although they remained α-helical in solution, lost the ability to form fibrils and were much less toxic to T cells than the wild type (37, 110). This suggested that cross-α amyloid formation plays a role in the pathogenicity of MRSA. At the molecular level, lipids were suggested to accelerate PSMα3 fibril formation (118), while a dynamic process of PSMα3 coaggregation with cell membranes potentially leads to massive T-cell deformation (37). Furthermore, mutagenesis analyses of PSMα3 indicated the role of positive charges, especially Lys17, in interactions with the membrane and suggested their regulation by inter- and intrahelical electrostatic interactions within the cross-α fibril (37). Overall, this mechanistic model suggests that PSMα3 cytotoxicity is governed not by a single entity (e.g., oligomers or mature fibrils) but rather by the ability to form cross-α fibrils that involves a dynamic process of coaggregation with the cell membrane, rupturing it (37). This might be relevant to amyloid toxicity in general, with the exact conformation that contributes to amyloid toxicity still under debate and which might be protein specific (124, 125).

Despite the stable formation of PSMα3 α-helices in solution, in fibrils and in crystals (37, 110), the tendency to form β-rich structures is embedded in its sequence, induced via mutation (37), or truncations (38). Some derivatives also show gain of antibacterial activity (38, 109). Specifically, the short six-residue segment PSM$\alpha3_{7-12}$ has antibacterial activity toward a few of Gram-positive bacteria, including *Staphylococcus hominis* but not against the secreting *S. aureus*. In contrast to the α-helical nature of the parent peptide, PSM$\alpha3_{7-12}$ was shown to form atypical and polymorphic β-rich fibrils, which are in variance from the cross-β canonical architecture (38). One polymorph was fundamentally different from typical cross-β steric zippers, displaying no dry interface between pairs of β-sheets. Instead, hexamers of β-sheets formed cylindrical channels running along the fibril-like structure, effectively yielding nanotubes (Fig. 3). The second polymorph was composed of out-of-register β-sheets (Fig. 3), meaning that unlike in canonical cross-β fibrils, β-strands are not perpendicular to the fibril axis (126, 127). Such an extreme polymorphism was exceptional within the hundreds of structures of amyloid-like spine segments solved to date (49).

Overall, the extreme structural polymorphism revealed in the *S. aureus* PSM family and their shorter derivatives, showing different amyloid fibrillar architectures, is attributed to their functional plasticity encompassing various activities of MRSA pathogenicity (38, 110).

THE SCAFFOLD AMYLOID CURLI SERVES AS AN IMMUNOMODULATOR

The curli fibers from *Enterobacteriaceae* are the foremost studied functional amyloids (128). Many commensal *E. coli* strains and the commonly studied lab strains express curli at temperatures of $<$30°C. In contrast, pathogenic *E. coli* strains like UPECs, EAECs including the 2012 German outbreak strain (sometimes grouped as an EHEC) and *S.* Typhimurium, have been shown to express curli at 37°C (62).

Curli regulation is under the control of two divergent operons *csgBAC* (curli-specific gene) and *csgDEFG*, with *csgD* encoding the transcriptional master regulator of both curli and cellulose (29). In *E. coli*, curli fibers compose up to 85% of the biofilm biomass (129), spatially expressed in the wrinkles of structured colonies (130) and often form an interwoven mesh that cradle the individual bacterial cell (54). The structural components of curli fibers are the self-assembling CsgA and CsgB, which are found at ratios of 20:1, respectively (131). These monomeric subunits self-assemble into oligomers, which then assemble into protofibrils before cross-assembling to form thicker mature fibrils (132). The rate of amyloid fibrillation of synthetic curli monomers was shown to be increased in the presence of eDNA (63). The width of curli fibers typically ranges between 4 and 10 nm, and they were suggested to adopt the typical cross-β architecture of amyloids, where the β-sheet strands are oriented perpendicular to the axis of the fiber. This construction provides a high degree of robustness and stability, along with exceptional resistance to sodium dodecyl sulfate and proteolytic treatments (29).

Like TasA, curli acts *in vitro* as an essential scaffold protein during biofilm formation. Curli mutants form less-structured colonies compared to a curli-only-producing strain, which is characterized by concentric rings radiating out from the center of the colony (54). A strain deficient in both curli and the exopolysaccharide cellulose (but none of the single mutants) forms completely featureless colonies, indicating that cellulose and curli interact (133).

Curli was shown to be expressed and active *in vivo* as well, since CsgA antibodies were detected in the blood of human sepsis patients (134) and curli expression was demonstrated for *S.* Typhimurium in the ceca and colons of mice (135). Furthermore, it was recently demonstrated that human monoclonal antibodies (3H3) with pan-amyloid epitope binding ability can disrupt and in conjunction with antibiotics to clear catheter-associated *S.* Typhimurium biofilms in mice. The antibody was shown to disrupt the biofilm structure both *in vitro* and *in vivo* by inhibiting curli polymerization (69). Interestingly, intermediate curli aggregates were more cytotoxic than mature curli fibrils to bone marrow-derived macrophages, similar to other cross-β amyloids which are mostly toxic in their oligomeric forms (132). When DNA is released during cell death, it facilitates fibrillation of curli into larger fibrillar structures (132).

In vivo, curli also directly regulates the immune system and is known to induce inflammation by activating the immune Toll-like receptors (TLRs) (136), a role of equal importance for host colonization. When this recognition occurs in the body outside the intestinal tract, it leads to a proinflammatory response with the activation of the production of cytokines, chemokines (interleukin-6/8), tumor necrosis factor alpha, and nitric oxide (134, 136). Indeed, mice exposed to curli in the gut were shown to have in increased levels of autoantibodies and joint inflammation (135). Curli-eDNA complexes were shown to be strong immune stimulators that activate both innate and adaptive immunity and trigger the production of autoantibodies (63) and TLR9 (136). The process takes advantage of the β-sheets structure of curli binding to the cell surface of TLR2, which leads to the internalization of the curli-eDNA complex into endosomes. Once internalized, the complex binds to endosomal TLR9, which induces type I interferon production and the subsequent production of autoantibodies (137). The recog-

nition of curli by TLR2 and the subsequent autoimmune response are abolished when the quaternary structure is disrupted via point mutations in *csgA* (137).

Similarly to curli, other microbial amyloids, including PSMs, and some human amyloids also activate TLRs and other immune receptors (138–142). This suggests the involvement of self-assembly in receptor activation in a sequence-nonspecific manner. Nevertheless, it is still unclear whether the structural recognition pattern of these receptors is indeed dependent on a particular form of protein self-assembly. It is possible that receptor activation is achieved with a very low concertation of the amyloids, involving soluble species and not mature fibrils.

In addition to their proinflammatory roles, curli amyloids can also induce an anti-inflammatory response when expressed by noninvasive strains (128). During chronic colonization, a curli-associated biofilm phenotype has emerged as a pathoadaptive trait associated with noninvasive phenotypes (143). For example, curli appears to be an antivirulence factor in acute systemic typhus-like infection in rodents caused by *S*. Typhimurium, blocking colonization of the spleen and other internal organs (144). Similarly, the recognition of amyloid fibers in enteric biofilms by the TLR 2/1 complex promotes an anti-inflammatory response and reinforce the barrier function by promoting intestinal epithelial integrity (145). Furthermore, the oral administration of curli fibers reduced severity of colitis in a mouse model for inflammatory bowel disease to the extent that the application of curli was suggested as potential treatment for intestinal inflammatory disorders (146). Overall, these collective results suggest that the spatial location of curli is of vital importance when it comes to the host's immune response, triggering a proinflammatory response in a systemic infection and an anti-inflammatory response when localized in the gut (147).

Treatment with curli proteins to reduce inflammation should be considered with great caution due to recent evidence for the existence of curli-induced α-synuclein pathology. Recently, it was shown that curli can accelerate α-synuclein (Parkinson's disease pathogenic amyloid) aggregation and that when human α-synuclein-overexpressing mice were colonized with curli-producing *E. coli* there was a marked increase in gut and brain pathology (148). However, this increased α-synuclein pathology is only witnessed in genetically predisposed mice (149). Therefore, curli alone is not enough to trigger the disease over a short period and requires other predisposing factors. CsgC and CsgE are chaperon-like proteins produced by *E. coli* which prevent the fibrilization of curli inside the *E. coli* cell. CsgC is also able to arrest fibrilization of α-synuclein, while CsgE actually accelerated the formation of α-synuclein amyloid *in vitro* (150). This neurotoxicity is in agreement with the pathologies of human neurodegenerative diseases, including Alzheimer's and Parkinson's, where the accumulation of amyloids and the local inflammation caused by the immune response to those amyloids contributes to the subsequent injury of tissue (151). However, the preliminary studies demonstrating that CsgC prevents the polymerization of amyloids seem to inspire interesting possibilities into the treatment of amyloid systemic infection and neurodegenerative disease merit further investigation.

CONCLUSIONS AND FUTURE DIRECTIONS: TARGETING FUNCTIONAL AMYLOIDS TO CONTROL MULTICELLULAR MICROBIAL COMMUNITIES

In this review, we have focused on the nonscaffold roles of amyloids in toxicity, signaling, and interaction with the immune system.

Signaling

In multicellular eukaryotes, numerous examples of ECM-driven signals crucial for the determination of both cell behavior and correct tissue morphogenesis have been described. There is no apparent reason to think that bacterial ECM would differ in this aspect. ECM-derived signals may be a common feature of cell communities surrounded by an extracellular matrix, as they allow for probing of the local environment and dynamic adjustment of signals (25). Evidence for potential regulatory activities of functional amyloids was recently published for TasA in *B. subtilis* (80, 93), although the

complete molecular mechanisms and whether TasA exerts its signal as an amyloid remain to be resolved. TasA was shown to regulate stress tolerance, motility, and ECM production independently of its scaffolding roles (80, 93). Additional evidence for TasA-driven regulation of gene expression rises from the spatial organization of different subpopulations in the biofilm, which are modified in amyloid mutants compared to the wild type, both in *B. subtilis* and in *E. coli* (54, 79). The defects in cell behavior can be explained as a lack of TasA-derived regulatory cues. It will be interesting to examine the possible role of the ECM and, specifically, microbial amyloids in the regulation of cell localization and genetic program activation in other species. Identifying with clarity the domains that account for these added functions and their specific inhibitors may be essential to extend affectively the repertoire of antivirulence drugs that modulate biofilm formation.

Toxins

Toxicity is also an essential feature for survival in complex environments. In nature, multiple species of bacteria compete for organic material, cluster with their own species, and form biofilms that compete for a niche with neighboring communities (152). The dual role of amyloids as toxins and scaffolds seems to directly address these complementary functions and increases the chances of the community as a whole to survive. It remains unknown whether the antimicrobial properties of TasA are related to its amyloid state (77). In the case of PSMs, while the involvement of PSMαs in biofilms is related to the formation of cross-β fibrils, which provide a stable scaffold (116), the cytotoxic activities against human cells of PSMα3 are probably related to the formation of cross-α fibrils (37, 38). Of note, toxic activity is not necessarily directly mediated via a specific toxic entity, such as monomers, oligomers, or fibrils, but via a dynamic process of coaggregation with the membrane (37). Toxic activities of PSMαs against bacterial cells might be mediated via different states and even by different secondary structures.

Immunomodulation

In addition to serving as scaffolds, regulators of gene expression, and toxins in the microbial domain, amyloids frequently interact with the eukaryote host of the microbial community. Indeed, microbial amyloids interact with the amyloids of host systems (153, 154), putatively providing some immune-evasive and survival strategies (155, 156), and have been suggested to contribute to the pathology of aggregation diseases (154, 155, 157–166). Curli fibers have been shown to enhance amyloid protein A (AA) amyloidosis in mice (167). AA amyloidosis is characterized by the buildup of amyloid protein A fibrils in tissue. Lundmark et al. demonstrated the ability of the bacterial curli to seed for fibril formation in mice leading to AA amyloidosis (167). The molecular structures shared between amyloids of different species may be involved in the creation of prion-like agents (168), raising concerns regarding the exposure of humans to various food sources and microbes that contain amyloids (169–171).

This review presents a number of studies demonstrating the pivotal nonscaffold roles of bacterial amyloid proteins. In *B. subtilis*, TasA acts as a toxin and adhesin and directly or indirectly regulates several developmental programs. In *S. aureus*, PSMs vary in their fibril architectures to allow discrete activities, as toxins and immune modulators. In *E. coli* and *S.* Typhimurium, curli serves as a main biofilm scaffolding fibril and also acts to promote or reduce inflammation, depending on its location. Many of these roles are proposed to be essential for the fitness of amyloid producers in several habitats. The different activities could be exerted by different fibril architectures, as clearly shown for the PSMs (Fig. 3); however, the same protein precursor may change its macro structure following interactions with membranes (172), hydrophobic substances (75), surfactants (173), or eDNA (64) and therefore function differently in different regions of the biofilm. Although PSMα3 is not observed within the biofilm biomass, its interactions with other surfaces within the host can affect its function. Correlating scaffold and nonscaffold activities with molecular structures seems to be the next frontier in the study of

microbial amyloids. It is early to determine whether we can target all different functions of microbial amyloids with the same compounds or aim for a cocktail of drugs against specific targets. Nonetheless, targeting virulent microbial amyloids might provide novel approaches to address the urgent need for therapeutics against resistant infections, since antivirulence drugs might reduce the aggressiveness of the infection while inducing lower resistance mechanisms compared to conventional bactericide antibiotics.

ACKNOWLEDGMENTS

The Kolodkin-Gal lab is supported by ISF-icore grant 152/1, Israel Science Foundation grant 119/16, Israel Foundation grant JSPS 184.20, Kamin grant by Israel Chief Scientist no. 67459, Israel Ministry of Science, Technology, & Space (grant 713454), the Ministry of Health (grant 713645), and the Angel-Fiavovich fund for ecological research. I.K.-G. is a recipient of Rowland and Sylvia Career Development Chair. The Landau lab is supported by the Israel Science Foundation (grant 560/16), the Israel Ministry of Science, Technology, & Space (grant 78567), and the U.S.-Israel Binational Science Foundation (grant 2017280).

REFERENCES

1. Harris KD, Kolodkin-Gal I. 2018. Applying the handicap principle to biofilms: condition-dependent signaling in *Bacillus subtilis* microbial communities. Environ Microbiol 21:531–540. https://doi.org/10.1111/1462-2920.14497.
2. Aguilar C, Vlamakis H, Losick R, Kolter R. 2007. Thinking about *Bacillus subtilis* as a multicellular organism. Curr Opin Microbiol 10:638–643. https://doi.org/10.1016/j.mib.2007.09.006.
3. Hall-Stoodley L, Stoodley P. 2009. Evolving concepts in biofilm infections. Cell Microbiol 11:1034–1043. https://doi.org/10.1111/j.1462-5822.2009.01323.x.
4. Costerton JW, Stewart PS, Greenberg EP. 1999. Bacterial biofilms: a common cause of persistent infections. Science 284:1318–1322. https://doi.org/10.1126/science.284.5418.1318.
5. Madhavan P, Jamal F, Pei CP, Othman F, Karunanidhi A, Ng KP. 2018. Comparative study of the effects of fluconazole and voriconazole on *Candida glabrata*, *Candida parapsilosis*, and *Candida rugosa* biofilms. Mycopathologia 183:499–511. https://doi.org/10.1007/s11046-018-0243-z.
6. Stewart PS, Costerton JW. 2001. Antibiotic resistance of bacteria in biofilms. Lancet 358:135–138. https://doi.org/10.1016/s0140-6736(01)05321-1.
7. Bryers JD. 2008. Medical biofilms. Biotechnol Bioeng 100:1–18. https://doi.org/10.1002/bit.21838.
8. Hill D, Rose B, Pajkos A, Robinson M, Bye P, Bell S, Elkins M, Thompson B, Macleod C, Aaron SD, Harbour C. 2005. Antibiotic susceptibilities of *Pseudomonas aeruginosa* isolates derived from patients with cystic fibrosis under aerobic, anaerobic, and biofilm conditions. J Clin Microbiol 43:5085–5090. https://doi.org/10.1128/JCM.43.10.5085-5090.2005.
9. Wilking JN, Zaburdaev V, De Volder M, Losick R, Brenner MP, Weitz DA. 2013. Liquid transport facilitated by channels in *Bacillus subtilis* biofilms. Proc Natl Acad Sci U S A 110:848–852. https://doi.org/10.1073/pnas.1216376110.
10. Dietrich LE, Okegbe C, Price-Whelan A, Sakhtah H, Hunter RC, Newman DK. 2013. Bacterial community morphogenesis is intimately linked to the intracellular redox state. J Bacteriol 195:1371–1380. https://doi.org/10.1128/JB.02273-12.
11. Bloom-Ackermann Z, Steinberg N, Rosenberg G, Oppenheimer-Shaanan Y, Pollack D, Ely S, Storzi N, Levy A, Kolodkin-Gal I. 2016. Toxin-antitoxin systems eliminate defective cells and preserve symmetry in *Bacillus subtilis* biofilms. Environ Microbiol 18:5032–5047. https://doi.org/10.1111/1462-2920.13471.
12. Kolodkin-Gal I, Elsholz AK, Muth C, Girguis PR, Kolter R, Losick R. 2013. Respiration control of multicellularity in *Bacillus subtilis* by a complex of the cytochrome chain with a membrane-embedded histidine kinase. Genes Dev 27:887–899. https://doi.org/10.1101/gad.215244.113.
13. Stewart PS, Franklin MJ. 2008. Physiological heterogeneity in biofilms. Nat Rev Microbiol 6:199–210. https://doi.org/10.1038/nrmicro1838.
14. Monds RD, O'Toole GA. 2009. The developmental model of microbial biofilms: ten years of a paradigm up for review. Trends Microbiol 17:73–87. https://doi.org/10.1016/j.tim.2008.11.001.
15. Serra DO, Hengge R. 2014. Stress responses go three dimensional: the spatial order of physiological differentiation in bacterial macrocolony biofilms. Environ Microbiol 16:1455–1471. https://doi.org/10.1111/1462-2920.12483.
16. Hassanov T, Karunker I, Steinberg N, Erez A, Kolodkin-Gal I. 2018. Novel antibiofilm chemotherapies target nitrogen from glutamate and glutamine. Sci Rep 8:7097. https://doi.org/10.1038/s41598-018-25401-z.
17. Asally M, Kittisopikul M, Rué P, Du Y, Hu Z, Çağatay T, Robinson AB, Lu H, Garcia-Ojalvo J, Süel GM. 2012. Localized cell death focuses mechanical forces during 3D patterning in a biofilm. Proc Natl Acad Sci U S A 109:18891–18896. https://doi.org/10.1073/pnas.1212429109.
18. Liu J, Prindle A, Humphries J, Gabalda-Sagarra M, Asally M, Lee DY, Ly S, Garcia-Ojalvo J, Suel GM. 2015. Metabolic co-dependence gives rise to collective oscillations within biofilms. Nature 523:550–554. https://doi.org/10.1038/nature14660.
19. O'Toole G, Kaplan HB, Kolter R. 2000. Biofilm formation as microbial development. Annu Rev Microbiol 54:49–79. https://doi.org/10.1146/annurev.micro.54.1.49.
20. Sauer K, Camper AK, Ehrlich GD, Costerton JW, Davies DG. 2002. *Pseudomonas aeruginosa* displays multiple phenotypes during development as a biofilm. J Bacteriol 184:1140–1154. https://doi.org/10.1128/jb.184.4.1140-1154.2002.
21. Southey-Pillig CJ, Davies DG, Sauer K. 2005. Characterization of temporal protein production in *Pseudomonas aeruginosa* biofilms. J Bacteriol 187:8114–8126. https://doi.org/10.1128/JB.187.23.8114-8126.2005.
22. Lopez D, Vlamakis H, Kolter R. 2009. Generation of multiple cell types in *Bacillus subtilis*. FEMS Microbiol Rev 33:152–163. https://doi.org/10.1111/j.1574-6976.2008.00148.x.
23. Xu KD, Stewart PS, Xia F, Huang CT, McFeters GA. 1998. Spatial physiological heterogeneity in *Pseudomonas aeruginosa* biofilm is determined by oxygen availability. Appl Environ Microbiol 64:4035–4039. https://doi.org/10.1128/AEM.64.10.4035-4039.1998.
24. Rani SA, Pitts B, Beyenal H, Veluchamy RA, Lewandowski Z, Davison WM, Buckingham-Meyer K, Stewart PS. 2007. Spatial patterns of DNA replication, protein synthesis, and oxygen concentration within bacterial biofilms reveal diverse physiological states. J Bacteriol 189:4223–4233. https://doi.org/10.1128/JB.00107-07.
25. Steinberg N, Kolodkin-Gal I. 2015. The matrix reloaded: probing the extracellular matrix synchronizes bacterial communities. J Bacteriol 197:2092–2103. https://doi.org/10.1128/JB.02516-14.
26. Branda SS, Vik S, Friedman L, Kolter R. 2005. Biofilms: the matrix revisited. Trends Microbiol 13:20–26. https://doi.org/10.1016/j.tim.2004.11.006.
27. Keren-Paz A, Cohen-Cymberknoh M, Kolodkin-Gal D, Karunker I, Dersch S, Wolf SG, Olender T, Kartvelishvily E, Kapishnikov S, Green-Zelinger P, Shteinberg M, Zamir G, Gal A, Graumann P, Kerem E, Kolodkin-Gal I. 2020. A novel calcium-concentrating compartment drives biofilm for-

mation and persistent infections. bioRxiv https://doi.org/10.1101/2020.01.08.898569:2020.01.08.898569.
28. Keren-Paz A, Kolodkin-Gal I. 2020. A brick in the wall: discovering a novel mineral component of the biofilm extracellular matrix. N Biotechnol 56:9–15. https://doi.org/10.1016/j.nbt.2019.11.002.
29. Chapman MR, Robinson LS, Pinkner JS, Roth R, Heuser J, Hammar M, Normark S, Hultgren SJ. 2002. Role of *Escherichia coli* curli operons in directing amyloid fiber formation. Science 295:851–855. https://doi.org/10.1126/science.1067484.
30. Schwartz K, Syed AK, Stephenson RE, Rickard AH, Boles BR. 2012. Functional amyloids composed of phenol-soluble modulins stabilize *Staphylococcus aureus* biofilms. PLoS Pathog 8:e1002744. https://doi.org/10.1371/journal.ppat.1002744.
31. Lembre P, Di Martino P, Vendrely C. 2014. Amyloid peptides derived from CsgA and FapC modify the viscoelastic properties of biofilm model matrices. Biofouling 30:415–426. https://doi.org/10.1080/08927014.2014.880112.
32. Romero D, Aguilar C, Losick R, Kolter R. 2010. Amyloid fibers provide structural integrity to *Bacillus subtilis* biofilms. Proc Natl Acad Sci U S A https://doi.org/10.1073/pnas.0910560107.
33. El Mammeri N, Hierrezuelo J, Tolchard J, Camara-Almiron J, Caro-Astorga J, Alvarez-Mena A, Dutour A, Berbon M, Shenoy J, Morvan E, Grelard A, Kauffmann B, Lecomte S, de Vicente A, Habenstein B, Romero D, Loquet A. 2019. Molecular architecture of bacterial amyloids in *Bacillus* biofilms. FASEB J 33:12146–12163. https://doi.org/10.1096/fj.201900831R.
34. Van Gerven N, Van der Verren SE, Reiter DM, Remaut H. 2018. The role of functional amyloids in bacterial virulence. J Mol Biol 430:3657–3684. https://doi.org/10.1016/j.jmb.2018.07.010.
35. Erskine E, Morris RJ, Schor M, Earl C, Gillespie RMC, Bromley KM, Sukhodub T, Clark L, Fyfe PK, Serpell LC, Stanley-Wall NR, MacPhee CE. 2018. Formation of functional, non-amyloidogenic fibres by recombinant *Bacillus subtilis* TasA. Mol Microbiol 110:897–913. https://doi.org/10.1111/mmi.13985.
36. Diehl A, Roske Y, Ball L, Chowdhury A, Hiller M, Moliere N, Kramer R, Stoppler D, Worth CL, Schlegel B, Leidert M, Cremer N, Erdmann N, Lopez D, Stephanowitz H, Krause E, van Rossum BJ, Schmieder P, Heinemann U, Turgay K, Akbey U, Oschkinat H. 2018. Structural changes of TasA in biofilm formation of *Bacillus subtilis*. Proc Natl Acad Sci U S A 115:3237–3242. https://doi.org/10.1073/pnas.1718102115.
37. Tayeb-Fligelman E, Salinas N, Tabachnikov O, Landau M. 2020. *Staphylococcus aureus* PSMα3 cross-α fibril polymorphism and determinants of cytotoxicity. Structure 28:301–313 e6. https://doi.org/10.1016/j.str.2019.12.006.
38. Salinas N, Colletier JP, Moshe A, Landau M. 2018. Extreme amyloid polymorphism in *Staphylococcus aureus* virulent PSMα peptides. Nat Commun 9:3512. https://doi.org/10.1038/s41467-018-05490-0.
39. Schmidt M, Rohou A, Lasker K, Yadav JK, Schiene-Fischer C, Fandrich M, Grigorieff N. 2015. Peptide dimer structure in an Aβ(1-42) fibril visualized with cryo-EM. Proc Natl Acad Sci U S A 112:11858–11863. https://doi.org/10.1073/pnas.1503455112.
40. Walti MA, Ravotti F, Arai H, Glabe CG, Wall JS, Bockmann A, Guntert P, Meier BH, Riek R. 2016. Atomic-resolution structure of a disease-relevant Aβ(1-42) amyloid fibril. Proc Natl Acad Sci U S A 113:E4976–E4984. https://doi.org/10.1073/pnas.1600749113.
41. Colvin MT, Silvers R, Ni QZ, Can TV, Sergeyev I, Rosay M, Donovan KJ, Michael B, Wall J, Linse S, Griffin RG. 2016. Atomic resolution structure of monomorphic Aβ42 amyloid fibrils. J Am Chem Soc 138:9663–9674. https://doi.org/10.1021/jacs.6b05129.
42. Xiao Y, Ma B, McElheny D, Parthasarathy S, Long F, Hoshi M, Nussinov R, Ishii Y. 2015. Aβ(1-42) fibril structure illuminates self-recognition and replication of amyloid in Alzheimer's disease. Nat Struct Mol Biol 22:499–505. https://doi.org/10.1038/nsmb.2991.
43. Paravastu AK, Leapman RD, Yau WM, Tycko R. 2008. Molecular structural basis for polymorphism in Alzheimer's β-amyloid fibrils. Proc Natl Acad Sci U S A 105:18349–18354. https://doi.org/10.1073/pnas.0806270105.
44. Gremer L, Scholzel D, Schenk C, Reinartz E, Labahn J, Ravelli RBG, Tusche M, Lopez-Iglesias C, Hoyer W, Heise H, Willbold D, Schroder GF. 2017. Fibril structure of amyloid-ss(1-42) by cryoelectron microscopy. Science 358:116–119. https://doi.org/10.1126/science.aao2825.
45. Fitzpatrick AWP, Falcon B, He S, Murzin AG, Murshudov G, Garringer HJ, Crowther RA, Ghetti B, Goedert M, Scheres SHW. 2017. Cryo-EM structures of tau filaments from Alzheimer's disease. Nature 547:185–190. https://doi.org/10.1038/nature23002.
46. Van Melckebeke H, Wasmer C, Lange A, Ab E, Loquet A, Bockmann A, Meier BH. 2010. Atomic-resolution three-dimensional structure of HET-s(218-289) amyloid fibrils by solid-state NMR spectroscopy. J Am Chem Soc 132:13765–13775. https://doi.org/10.1021/ja104213j.
47. Chen SW, Drakulic S, Deas E, Ouberai M, Aprile FA, Arranz R, Ness S, Roodveldt C, Guilliams T, De-Genst EJ, Klenerman D, Wood NW, Knowles TP, Alfonso C, Rivas G, Abramov AY, Valpuesta JM, Dobson CM, Cremades N. 2015. Structural characterization of toxic oligomers that are kinetically trapped during alpha-synuclein fibril formation. Proc Natl Acad Sci U S A 112:E1994–E2003. https://doi.org/10.1073/pnas.1421204112.
48. Sawaya MR, Sambashivan S, Nelson R, Ivanova MI, Sievers SA, Apostol MI, Thompson MJ, Balbirnie M, Wiltzius JJ, McFarlane HT, Madsen AO, Riekel C, Eisenberg D. 2007. Atomic structures of amyloid cross-β spines reveal varied steric zippers. Nature 447:453–457. https://doi.org/10.1038/nature05695.
49. Eisenberg DS, Sawaya MR. 2017. Structural studies of amyloid proteins at the molecular level. Annu Rev Biochem 86:69–95. https://doi.org/10.1146/annurev-biochem-061516-045104.
50. Close W, Neumann M, Schmidt A, Hora M, Annamalai K, Schmidt M, Reif B, Schmidt V, Grigorieff N, Fandrich M. 2018. Physical basis of amyloid fibril polymorphism. Nat Commun 9:699. https://doi.org/10.1038/s41467-018-03164-5.
51. Nicastro L, Tukel C. 2019. Bacterial amyloids: the link between bacterial infections and autoimmunity. Trends Microbiol 27:954–963. https://doi.org/10.1016/j.tim.2019.07.002.
52. Hufnagel DA, Tukel C, Chapman MR. 2013. Disease to dirt: the biology of microbial amyloids. PLoS Pathog 9:e1003740. https://doi.org/10.1371/journal.ppat.1003740.
53. Dueholm MS, Petersen SV, Sønderkær M, Larsen P, Christiansen G, Hein KL, Enghild JJ, Nielsen JL, Nielsen KL, Nielsen PH, Otzen DE. 2010. Functional amyloid in *Pseudomonas*. Mol Microbiol 77:1009–1020. https://doi.org/10.1111/j.1365-2958.2010.07269.x.
54. Serra DO, Richter AM, Klauck G, Mika F, Hengge R. 2013. Microanatomy at cellular resolution and spatial order of physiological differentiation in a bacterial biofilm. mBio 4:e00103-13. https://doi.org/10.1128/mBio.00103-13.
55. Angelini TE, Roper M, Kolter R, Weitz DA, Brenner MP. 2009. *Bacillus subtilis* spreads by surfing on waves of surfactant. Proc Natl Acad Sci U S A 106:18109–18113. https://doi.org/10.1073/pnas.0905890106.
56. Cairns LS, Hobley L, Stanley-Wall NR. 2014. Biofilm formation by *Bacillus subtilis*: new insights into regulatory strategies and assembly mechanisms. Mol Microbiol 93:587–598. https://doi.org/10.1111/mmi.12697.
57. Kempes CP, Okegbe C, Mears-Clarke Z, Follows MJ, Dietrich LE. 2014. Morphological optimization for access to dual oxidants in biofilms. Proc Natl Acad Sci U S A 111:208–213. https://doi.org/10.1073/pnas.1315521110.
58. Okshevsky M, Meyer RL. 2013. The role of extracellular DNA in the establishment, maintenance, and perpetuation of bacterial biofilms. Crit Rev Microbiol 41:341–352. https://doi.org/10.3109/1040841X.2013.841639.
59. Das T, Sehar S, Manefield M. 2013. The roles of extracellular DNA in the structural integrity of extracellular polymeric substance and bacterial biofilm development. Environ Microbiol Rep 5:778–786. https://doi.org/10.1111/1758-2229.12085.
60. Deleault NR, Harris BT, Rees JR, Supattapone S. 2007. Formation of native prions from minimal components *in vitro*. Proc Natl Acad Sci U S A 104:9741–9746. https://doi.org/10.1073/pnas.0702662104.
61. Barrantes A, Rejas MT, Benitez MJ, Jimenez JS. 2007. Interaction between Alzheimer's Aβ1-42 peptide and DNA detected by surface plasmon resonance. J Alzheimers Dis 12:345–355. https://doi.org/10.3233/jad-2007-12408.
62. Tursi SA, Tukel C. 2018. Curli-containing enteric biofilms inside and out: matrix composition, immune recognition, and disease implications. Microbiol Mol Biol Rev 82:e00028-18. https://doi.org/10.1128/MMBR.00028-18.
63. Gallo PM, Rapsinski GJ, Wilson RP, Oppong GO, Sriram U, Goulian M, Buttaro B, Caricchio R, Gallucci S, Tukel C. 2015. Amyloid-DNA composites of bacterial biofilms stimulate autoimmunity. Immunity 42:1171–1184. https://doi.org/10.1016/j.immuni.2015.06.002.
64. Schwartz K, Ganesan M, Payne DE, Solomon MJ, Boles BR. 2016. Extracellular DNA facilitates the formation of functional amyloids in *Staphylococcus aureus* biofilms. Mol Microbiol 99:123–134. https://doi.org/10.1111/mmi.13219.
65. Dragos A, Kiesewalter H, Martin M, Hsu CY, Hartmann R, Wechsler T,

Eriksen C, Brix S, Drescher K, Stanley-Wall N, Kummerli R, Kovacs AT. 2018. Division of labor during biofilm matrix production. Curr Biol 28:1903–1913 e5. https://doi.org/10.1016/j.cub.2018.04.046.
66. Taglialegna A, Lasa I, Valle J. 2016. Amyloid structures as biofilm matrix scaffolds. J Bacteriol 198:2579–2588. https://doi.org/10.1128/JB.00122-16.
67. Perov S, Lidor O, Salinas N, Golan N, Tayeb-Fligelman E, Deshmukh M, Willbold D, Landau M. 2019. Structural insights into curli CsgA cross-β fibril architecture inspired repurposing of anti-amyloid compounds as anti-biofilm agents. bioRxiv https://doi.org/10.1101/493668:493668.
68. Cegelski L, Pinkner JS, Hammer ND, Cusumano CK, Hung CS, Chorell E, Aberg V, Walker JN, Seed PC, Almqvist F, Chapman MR, Hultgren SJ. 2009. Small-molecule inhibitors target *Escherichia coli* amyloid biogenesis and biofilm formation. Nat Chem Biol 5:913–919. https://doi.org/10.1038/nchembio.242.
69. Tursi SA, Puligedda RD, Szabo P, Nicastro LK, Miller AL, Qiu C, Gallucci S, Relkin NR, Buttaro BA, Dessain SK, Tukel C. 2020. *Salmonella* Typhimurium biofilm disruption by a human antibody that binds a pan-amyloid epitope on curli. Nat Commun 11:1007. https://doi.org/10.1038/s41467-020-14685-3.
70. Jones D. 1988. The place of *Listeria* among Gram-positive bacteria. Infection 16:S85–S88. https://doi.org/10.1007/BF01639727.
71. Logan NA. 2012. Bacillus and relatives in foodborne illness. J Appl Microbiol 112:417–429. https://doi.org/10.1111/j.1365-2672.2011.05204.x.
72. Hou Q, Kolodkin-Gal I. 2020. Harvesting the complex pathways of antibiotic production and resistance of soil bacilli for optimizing plant microbiome. FEMS Microbiol Ecol 96:fiaa142. https://doi.org/10.1093/femsec/fiaa142.
73. Romero D, Vlamakis H, Losick R, Kolter R. 2011. An accessory protein required for anchoring and assembly of amyloid fibres in B. subtilis biofilms. Mol Microbiol 80:1155–1168. https://doi.org/10.1111/j.1365-2958.2011.07653.x.
74. Branda SS, Chu F, Kearns DB, Losick R, Kolter R. 2006. A major protein component of the *Bacillus subtilis* biofilm matrix. Mol Microbiol 59:1229–1238. https://doi.org/10.1111/j.1365-2958.2005.05020.x.
75. Chai L, Romero D, Kayatekin C, Akabayov B, Vlamakis H, Losick R, Kolter R. 2013. Isolation, characterization, and aggregation of a structured bacterial matrix precursor. J Biol Chem 288:17559–17568. https://doi.org/10.1074/jbc.M113.453605.
76. Bucher T, Oppenheimer-Shaanan Y, Savidor A, Bloom-Ackermann Z, Kolodkin-Gal I. 2015. Disturbance of the bacterial cell wall specifically interferes with biofilm formation. Environ Microbiol Rep 7:990–1004. https://doi.org/10.1111/1758-2229.12346.
77. Stover AG, Driks A. 1999. Secretion, localization, and antibacterial activity of TasA, a *Bacillus subtilis* spore-associated protein. J Bacteriol 181:1664–1672. https://doi.org/10.1128/JB.181.5.1664-1672.1999.
78. Bucciantini M, Giannoni E, Chiti F, Baroni F, Formigli L, Zurdo J, Taddei N, Ramponi G, Dobson CM, Stefani M. 2002. Inherent toxicity of aggregates implies a common mechanism for protein misfolding diseases. Nature 416:507–511. https://doi.org/10.1038/416507a.
79. Vlamakis H, Aguilar C, Losick R, Kolter R. 2008. Control of cell fate by the formation of an architecturally complex bacterial community. Genes Dev 22:945–953. https://doi.org/10.1101/gad.1645008.
80. Steinberg N, Keren-Paz A, Hou Q, Doron S, Yanuka-Golub K, Olender T, Hadar R, Rosenberg G, Jain R, Camara-Almiron J, Romero D, van Teeffelen S, Kolodkin-Gal I. 2020. The extracellular matrix protein TasA is a developmental cue that maintains a motile subpopulation within *Bacillus subtilis* biofilms. Sci Signal 13:eaaw8905. https://doi.org/10.1126/scisignal.aaw8905.
81. Chai Y, Kolter R, Losick R. 2010. Reversal of an epigenetic switch governing cell chaining in *Bacillus subtilis* by protein instability. Mol Microbiol 78:218–229. https://doi.org/10.1111/j.1365-2958.2010.07335.x.
82. Cozy LM, Phillips AM, Calvo RA, Bate AR, Hsueh YH, Bonneau R, Eichenberger P, Kearns DB. 2012. SlrA/SinR/SlrR inhibits motility gene expression upstream of a hypersensitive and hysteretic switch at the level of sigma(D) in *Bacillus subtilis*. Mol Microbiol 83:1210–1228. https://doi.org/10.1111/j.1365-2958.2012.08003.x.
83. Kobayashi K. 2008. SlrR/SlrA controls the initiation of biofilm formation in *Bacillus subtilis*. Mol Microbiol 69:1399–1410. https://doi.org/10.1111/j.1365-2958.2008.06369.x.
84. Newman JA, Lewis RJ. 2013. Exploring the role of SlrR and SlrA in the SinR epigenetic switch. Commun Integr Biol 6:e25658. https://doi.org/10.4161/cib.25658.
85. Chai Y, Norman T, Kolter R, Losick R. 2010. An epigenetic switch governing daughter cell separation in *Bacillus subtilis*. Genes Dev 24:754–765. https://doi.org/10.1101/gad.1915010.
86. Kearns DB, Chu F, Branda SS, Kolter R, Losick R. 2005. A master regulator for biofilm formation by *Bacillus subtilis*. Mol Microbiol 55:739–749. https://doi.org/10.1111/j.1365-2958.2004.04440.x.
87. Bai U, Mandic-Mulec I, Smith I. 1993. SinI modulates the activity of SinR, a developmental switch protein of *Bacillus subtilis*, by protein-protein interaction. Genes Dev 7:139–148. https://doi.org/10.1101/gad.7.1.139.
88. Lewis RJ, Brannigan JA, Smith I, Wilkinson AJ. 1996. Crystallization of the *Bacillus subtilis* sporulation inhibitor SinR, complexed with its antagonist, SinI. FEBS Lett 378:98–100. https://doi.org/10.1016/0014-5793(95)01432-2.
89. Kearns DB. 2010. A field guide to bacterial swarming motility. Nat Rev Microbiol 8:634–644. https://doi.org/10.1038/nrmicro2405.
90. Steinberg N, Rosenberg G, Keren-Paz A, Kolodkin-Gal I. 2018. Collective vortex-like movement of *Bacillus subtilis* facilitates the generation of floating biofilms. Front Microbiol 9:590. https://doi.org/10.3389/fmicb.2018.00590.
91. van Gestel J, Vlamakis H, Kolter R. 2015. From cell differentiation to cell collectives: *Bacillus subtilis* uses division of labor to migrate. PLoS Biol 13:e1002141. https://doi.org/10.1371/journal.pbio.1002141.
92. Grau RR, de Ona P, Kunert M, Lenini C, Gallegos-Monterrosa R, Mhatre E, Vileta D, Donato V, Holscher T, Boland W, Kuipers OP, Kovacs AT. 2015. A duo of potassium-responsive histidine kinases govern the multicellular destiny of *Bacillus subtilis*. mBio 6:e00581-15. https://doi.org/10.1128/mBio.00581-15.
93. Camara-Almiron J, Navarro Y, Diaz-Martinez L, Magno-Perez-Bryan MC, Molina-Santiago C, Pearson JR, de Vicente A, Perez-Garcia A, Romero D. 2020. Dual functionality of the amyloid protein TasA in *Bacillus* physiology and fitness on the phylloplane. Nat Commun 11:1859. https://doi.org/10.1038/s41467-020-15758-z.
94. Dwyer DJ, Belenky PA, Yang JH, MacDonald IC, Martell JD, Takahashi N, Chan CT, Lobritz MA, Braff D, Schwarz EG, Ye JD, Pati M, Vercruysse M, Ralifo PS, Allison KR, Khalil AS, Ting AY, Walker GC, Collins JJ. 2014. Antibiotics induce redox-related physiological alterations as part of their lethality. Proc Natl Acad Sci U S A 111:E2100–E2109. https://doi.org/10.1073/pnas.1401876111.
95. Dwyer DJ, Kohanski MA, Collins JJ. 2009. Role of reactive oxygen species in antibiotic action and resistance. Curr Opin Microbiol 12:482–489. https://doi.org/10.1016/j.mib.2009.06.018.
96. Hyyrylainen HL, Bolhuis A, Darmon E, Muukkonen L, Koski P, Vitikainen M, Sarvas M, Pragai Z, Bron S, van Dijl JM, Kontinen VP. 2001. A novel two-component regulatory system in *Bacillus subtilis* for the survival of severe secretion stress. Mol Microbiol 41:1159–1172. https://doi.org/10.1046/j.1365-2958.2001.02576.x.
97. Noone D, Botella E, Butler C, Hansen A, Jende I, Devine KM. 2012. Signal perception by the secretion stress-responsive CssRS two-component system in *Bacillus subtilis*. J Bacteriol 194:1800–1814. https://doi.org/10.1128/JB.05767-11.
98. Westers H, Westers L, Darmon E, van Dijl JM, Quax WJ, Zanen G. 2006. The CssRS two-component regulatory system controls a general secretion stress response in *Bacillus subtilis*. FEBS J 273:3816–3827. https://doi.org/10.1111/j.1742-4658.2006.05389.x.
99. Darmon E, Noone D, Masson A, Bron S, Kuipers OP, Devine KM, van Dijl JM. 2002. A novel class of heat and secretion stress-responsive genes is controlled by the autoregulated CssRS two-component system of *Bacillus subtilis*. J Bacteriol 184:5661–5671. https://doi.org/10.1128/jb.184.20.5661-5671.2002.
100. Zahrl D, Wagner M, Bischof K, Koraimann G. 2006. Expression and assembly of a functional type IV secretion system elicit extracytoplasmic and cytoplasmic stress responses in *Escherichia coli*. J Bacteriol 188:6611–6621. https://doi.org/10.1128/JB.00632-06.
101. Qin B, Fei C, Bridges AA, Mashruwala AA, Stone HA, Wingreen NS, Bassler BL. 2020. Cell position fates and collective fountain flow in bacterial biofilms revealed by light-sheet microscopy. Science 369:71–77. https://doi.org/10.1126/science.abb8501.
102. Craft KM, Nguyen JM, Berg LJ, Townsend SD. 2019. Methicillin-resistant *Staphylococcus aureus* (MRSA): antibiotic-resistance and the biofilm phenotype. Medchemcomm 10:1231–1241. https://doi.org/10.1039/c9md00044e.
103. Voyich JM, Braughton KR, Sturdevant DE, Whitney AR, Said-Salim B, Porcella SF, Long RD, Dorward DW, Gardner DJ, Kreiswirth BN, Musser JM, DeLeo FR. 2005. Insights into mechanisms used by *Staphylococcus*

aureus to avoid destruction by human neutrophils. J Immunol 175:3907–3919. https://doi.org/10.4049/jimmunol.175.6.3907.
104. Chatterjee SS, Joo H-S, Duong AC, Dieringer TD, Tan VY, Song Y, Fischer ER, Cheung GYC, Li M, Otto M. 2013. Essential *Staphylococcus aureus* toxin export system. Nat Med 19:364–367. https://doi.org/10.1038/nm.3047.
105. Wang R, Braughton KR, Kretschmer D, Bach T-HL, Queck SY, Li M, Kennedy AD, Dorward DW, Klebanoff SJ, Peschel A, Deleo FR, Otto M. 2007. Identification of novel cytolytic peptides as key virulence determinants for community-associated MRSA. Nat Med 13:1510–1514. https://doi.org/10.1038/nm1656.
106. Kretschmer D, Gleske AK, Rautenberg M, Wang R, Koberle M, Bohn E, Schoneberg T, Rabiet MJ, Boulay F, Klebanoff SJ, van Kessel KA, van Strijp JA, Otto M, Peschel A. 2010. Human formyl peptide receptor 2 senses highly pathogenic *Staphylococcus aureus*. Cell Host Microbe 7:463–473. https://doi.org/10.1016/j.chom.2010.05.012.
107. Cheung GY, Duong AC, Otto M. 2012. Direct and synergistic hemolysis caused by *Staphylococcus* phenol-soluble modulins: implications for diagnosis and pathogenesis. Microbes Infect 14:380–386. https://doi.org/10.1016/j.micinf.2011.11.013.
108. Cheung GY, Joo HS, Chatterjee SS, Otto M. 2014. Phenol-soluble modulins: critical determinants of staphylococcal virulence. FEMS Microbiol Rev 38:698–719. https://doi.org/10.1111/1574-6976.12057.
109. Cheung GY, Kretschmer D, Queck SY, Joo HS, Wang R, Duong AC, Nguyen TH, Bach TH, Porter AR, DeLeo FR, Peschel A, Otto M. 2014. Insight into structure-function relationship in phenol-soluble modulins using an alanine screen of the phenol-soluble modulin (PSM) α3 peptide. FASEB J 28:153–161. https://doi.org/10.1096/fj.13-232041.
110. Tayeb-Fligelman E, Tabachnikov O, Moshe A, Goldshmidt-Tran O, Sawaya MR, Coquelle N, Colletier JP, Landau M. 2017. The cytotoxic *Staphylococcus aureus* PSMα3 reveals a cross-α amyloid-like fibril. Science 355:831–833. https://doi.org/10.1126/science.aaf4901.
111. Mehlin C, Headley CM, Klebanoff SJ. 1999. An inflammatory polypeptide complex from *Staphylococcus epidermidis*: isolation and characterization. J Exp Med 189:907–918. https://doi.org/10.1084/jem.189.6.907.
112. McKevitt AI, Bjornson GL, Mauracher CA, Scheifele DW. 1990. Amino acid sequence of a deltalike toxin from *Staphylococcus epidermidis*. Infect Immun 58:1473–1475. https://doi.org/10.1128/IAI.58.5.1473-1475.1990.
113. Joo HS, Cheung GY, Otto M. 2011. Antimicrobial activity of community-associated methicillin-resistant *Staphylococcus aureus* is caused by phenol-soluble modulin derivatives. J Biol Chem 286:8933–8940. https://doi.org/10.1074/jbc.M111.221382.
114. Gonzalez DJ, Okumura CY, Hollands A, Kersten R, Akong-Moore K, Pence MA, Malone CL, Derieux J, Moore BS, Horswill AR, Dixon JE, Dorrestein PC, Nizet V. 2012. Novel phenol-soluble modulin derivatives in community-associated methicillin-resistant *Staphylococcus aureus* identified through imaging mass spectrometry. J Biol Chem 287:13889–13898. https://doi.org/10.1074/jbc.M112.349860.
115. Gonzalez DJ, Vuong L, Gonzalez IS, Keller N, McGrosso D, Hwang JH, Hung J, Zinkernagel A, Dixon JE, Dorrestein PC, Nizet V. 2014. Phenol soluble modulin (PSM) variants of community-associated methicillin-resistant *Staphylococcus aureus* (MRSA) captured using mass spectrometry-based molecular networking. Mol Cell Proteomics 13:1262–1272. https://doi.org/10.1074/mcp.M113.031336.
116. Deplanche M, Filho RA, Alekseeva L, Ladier E, Jardin J, Henry G, Azevedo V, Miyoshi A, Beraud L, Laurent F, Lina G, Vandenesch F, Steghens JP, Le Loir Y, Otto M, Gotz F, Berkova N. 2015. Phenol-soluble modulin alpha induces G_2/M phase transition delay in eukaryotic HeLa cells. FASEB J 29:1950–1959. https://doi.org/10.1096/fj.14-260513.
117. Jang KS, Park M, Lee JY, Kim JS. 2017. Mass spectrometric identification of phenol-soluble modulins in the ATCC(R) 43300 standard strain of methicillin-resistant *Staphylococcus aureus* harboring two distinct phenotypes. Eur J Clin Microbiol Infect Dis 36:1151–1157. https://doi.org/10.1007/s10096-017-2902-2.
118. Malishev R, Tayeb-Fligelman E, David S, Meijler MM, Landau M, Jelinek R. 2018. Reciprocal interactions between membrane bilayers and *Staphylococcus aureus* PSMα3 cross-α amyloid fibrils account for species-specific cytotoxicity. J Mol Biol 430:1431–1441. https://doi.org/10.1016/j.jmb.2018.03.022.
119. Periasamy S, Joo HS, Duong AC, Bach TH, Tan VY, Chatterjee SS, Cheung GY, Otto M. 2012. How *Staphylococcus aureus* biofilms develop their characteristic structure. Proc Natl Acad Sci U S A 109:1281–1286. https://doi.org/10.1073/pnas.1115006109.
120. Otto M. 2008. Staphylococcal biofilms. Curr Top Microbiol Immunol 322:207–228. https://doi.org/10.1007/978-3-540-75418-3_10.
121. Kobayashi SD, Braughton KR, Palazzolo-Ballance AM, Kennedy AD, Sampaio E, Kristosturyan E, Whitney AR, Sturdevant DE, Dorward DW, Holland SM, Kreiswirth BN, Musser JM, DeLeo FR. 2010. Rapid neutrophil destruction following phagocytosis of *Staphylococcus aureus*. J Innate Immun 2:560–575. https://doi.org/10.1159/000317134.
122. Cassat JE, Hammer ND, Campbell JP, Benson MA, Perrien DS, Mrak LN, Smeltzer MS, Torres VJ, Skaar EP. 2013. A secreted bacterial protease tailors the *Staphylococcus aureus* virulence repertoire to modulate bone remodeling during osteomyelitis. Cell Host Microbe 13:759–772. https://doi.org/10.1016/j.chom.2013.05.003.
123. Laabei M, Jamieson WD, Yang Y, van den Elsen J, Jenkins AT. 2014. Investigating the lytic activity and structural properties of *Staphylococcus aureus* phenol soluble modulin (PSM) peptide toxins. Biochim Biophys Acta 1838:3153–3161. https://doi.org/10.1016/j.bbamem.2014.08.026.
124. Stefani M. 2012. Structural features and cytotoxicity of amyloid oligomers: implications in Alzheimer's disease and other diseases with amyloid deposits. Prog Neurobiol 99:226–245. https://doi.org/10.1016/j.pneurobio.2012.03.002.
125. Ghosh D, Singh PK, Sahay S, Jha NN, Jacob RS, Sen S, Kumar A, Riek R, Maji SK. 2015. Structure based aggregation studies reveal the presence of helix-rich intermediate during alpha-synuclein aggregation. Sci Rep 5:9228. https://doi.org/10.1038/srep09228.
126. Liu C, Zhao M, Jiang L, Cheng PN, Park J, Sawaya MR, Pensalfini A, Gou D, Berk AJ, Glabe CG, Nowick J, Eisenberg D. 2012. Out-of-register beta-sheets suggest a pathway to toxic amyloid aggregates. Proc Natl Acad Sci U S A 109:20913–20918. https://doi.org/10.1073/pnas.1218792109.
127. Soriaga AB, Sangwan S, Macdonald R, Sawaya MR, Eisenberg D. 2016. Crystal structures of IAPP amyloidogenic segments reveal a novel packing motif of out-of-register beta sheets. J Phys Chem B 120:5810–5816. https://doi.org/10.1021/acs.jpcb.5b09981.
128. Blanco LP, Evans ML, Smith DR, Badtke MP, Chapman MR. 2012. Diversity, biogenesis, and function of microbial amyloids. Trends Microbiol 20:66–73. https://doi.org/10.1016/j.tim.2011.11.005.
129. Hung C, Zhou YZ, Pinkner JS, Dodson KW, Crowley JR, Heuser J, Chapman MR, Hadjifrangiskou M, Henderson JP, Hultgren SJ. 2013. *Escherichia coli* biofilms have an organized and complex extracellular matrix structure. mBio 4:e00645-13. https://doi.org/10.1128/mBio.00645-13.
130. DePas WH, Hufnagel DA, Lee JS, Blanco LP, Bernstein HC, Fisher ST, James GA, Stewart PS, Chapman MR. 2013. Iron induces bimodal population development by *Escherichia coli*. Proc Natl Acad Sci U S A 110:2629–2634. https://doi.org/10.1073/pnas.1218703110.
131. White AP, Collinson SK, Banser PA, Gibson DL, Paetzel M, Strynadka NC, Kay WW. 2001. Structure and characterization of AgfB from *Salmonella enteritidis* thin aggregative fimbriae. J Mol Biol 311:735–749. https://doi.org/10.1006/jmbi.2001.4876.
132. Nicastro LK, Tursi SA, Le LS, Miller AL, Efimov A, Buttaro B, Tam V, Tukel C. 2019. Cytotoxic curli intermediates form during salmonella biofilm development. J Bacteriol 201:e00095-19. https://doi.org/10.1128/JB.00095-19.
133. Thongsomboon W, Serra DO, Possling A, Hadjineophytou C, Hengge R, Cegelski L. 2018. Phosphoethanolamine cellulose: a naturally produced chemically modified cellulose. Science 359:334–338. https://doi.org/10.1126/science.aao4096.
134. Bian Z, Brauner A, Li Y, Normark S. 2000. Expression of and cytokine activation by *Escherichia coli* curli fibers in human sepsis. J Infect Dis 181:602–612. https://doi.org/10.1086/315233.
135. Miller AL, Pasternak JA, Medeiros NJ, Nicastro LK, Tursi SA, Hansen EG, Krochak R, Sokaribo AS, MacKenzie KD, Palmer MB, Herman DJ, Watson NL, Zhang Y, Wilson HL, Wilson RP, White AP, Tukel C. 2020. *In vivo* synthesis of bacterial amyloid curli contributes to joint inflammation during *S.* Typhimurium infection. PLoS Pathog 16:e1008591. https://doi.org/10.1371/journal.ppat.1008591.
136. Tukel C, Wilson RP, Nishimori JH, Pezeshki M, Chromy BA, Baumler AJ. 2009. Responses to amyloids of microbial and host origin are mediated through Toll-like receptor 2. Cell Host Microbe 6:45–53. https://doi.org/10.1016/j.chom.2009.05.020.
137. Tursi SA, Lee EY, Medeiros NJ, Lee MH, Nicastro LK, Buttaro B, Gallucci S, Wilson RP, Wong GCL, Tukel C. 2017. Bacterial amyloid curli acts as a carrier for DNA to elicit an autoimmune response via

TLR2 and TLR9. PLoS Pathog 13:e1006315. https://doi.org/10.1371/journal.ppat.1006315.
138. Rautenberg M, Joo HS, Otto M, Peschel A. 2011. Neutrophil responses to staphylococcal pathogens and commensals via the formyl peptide receptor 2 relates to phenol-soluble modulin release and virulence. FASEB J 25:1254–1263. https://doi.org/10.1096/fj.10-175208.
139. Migeotte I, Communi D, Parmentier M. 2006. Formyl peptide receptors: a promiscuous subfamily of G protein-coupled receptors controlling immune responses. Cytokine Growth Factor Rev 17:501–519. https://doi.org/10.1016/j.cytogfr.2006.09.009.
140. Le Y, Gong W, Tiffany HL, Tumanov A, Nedospasov S, Shen W, Dunlop NM, Gao JL, Murphy PM, Oppenheim JJ, Wang JM. 2001. Amyloid (beta)42 activates a G-protein-coupled chemoattractant receptor, FPR-like-1. J Neurosci 21:Rc123. https://doi.org/10.1523/JNEUROSCI.21-02-j0003.2001.
141. Liang TS, Wang JM, Murphy PM, Gao JL. 2000. Serum amyloid A is a chemotactic agonist at FPR2, a low-affinity *N*-formylpeptide receptor on mouse neutrophils. Biochem Biophys Res Commun 270:331–335. https://doi.org/10.1006/bbrc.2000.2416.
142. Schwartz K, Boles BR. 2013. Microbial amyloids: functions and interactions within the host. Curr Opin Microbiol 16:93–99. https://doi.org/10.1016/j.mib.2012.12.001.
143. MacKenzie KD, Palmer MB, Koster WL, White AP. 2017. Examining the link between biofilm formation and the ability of pathogenic *Salmonella* strains to colonize multiple host species. Front Vet Sci 4:138. https://doi.org/10.3389/fvets.2017.00138.
144. Fabrega A, Vila J. 2013. *Salmonella enterica* serovar Typhimurium skills to succeed in the host: virulence and regulation. Clin Microbiol Rev 26:308–341. https://doi.org/10.1128/CMR.00066-12.
145. Rakoff-Nahoum S, Paglino J, Eslami-Varzaneh F, Edberg S, Medzhitov R. 2004. Recognition of commensal microflora by Toll-like receptors is required for intestinal homeostasis. Cell 118:229–241. https://doi.org/10.1016/j.cell.2004.07.002.
146. Oppong GO, Rapsinski GJ, Tursi SA, Biesecker SG, Klein-Szanto AJP, Goulian M, McCauley C, Healy C, Wilson RP, Tukel C. 2015. Biofilm-associated bacterial amyloids dampen inflammation in the gut: oral treatment with curli fibres reduces the severity of hapten-induced colitis in mice. NPJ Biofilms Microbiomes 1:15019. https://doi.org/10.1038/npjbiofilms.2015.19.
147. Oppong GO, Rapsinski GJ, Newman TN, Nishimori JH, Biesecker SG, Tukel C. 2013. Epithelial cells augment barrier function via activation of the Toll-like receptor 2/phosphatidylinositol 3-kinase pathway upon recognition of *Salmonella enterica* serovar Typhimurium curli fibrils in the gut. Infect Immun 81:478–486. https://doi.org/10.1128/IAI.00453-12.
148. Sampson TR, Challis C, Jain N, Moiseyenko A, Ladinsky MS, Shastri GG, Thron T, Needham BD, Horvath I, Debelius JW, Janssen S, Knight R, Wittung-Stafshede P, Gradinaru V, Chapman M, Mazmanian SK. 2020. A gut bacterial amyloid promotes alpha-synuclein aggregation and motor impairment in mice. Elife 9:e53111. https://doi.org/10.7554/eLife.53111.
149. Chen SG, Stribinskis V, Rane MJ, Demuth DR, Gozal E, Roberts AM, Jagadapillai R, Liu R, Choe K, Shivakumar B, Son F, Jin S, Kerber R, Adame A, Masliah E, Friedland RP. 2016. Exposure to the functional bacterial amyloid protein curli enhances alpha-synuclein aggregation in aged Fischer 344 rats and *Caenorhabditis elegans*. Sci Rep 6:34477. https://doi.org/10.1038/srep34477.
150. Evans ML, Chorell E, Taylor JD, Aden J, Gotheson A, Li F, Koch M, Sefer L, Matthews SJ, Wittung-Stafshede P, Almqvist F, Chapman MR. 2015. The bacterial curli system possesses a potent and selective inhibitor of amyloid formation. Mol Cell 57:445–455. https://doi.org/10.1016/j.molcel.2014.12.025.
151. Guo JT, Yu J, Grass D, de Beer FC, Kindy MS. 2002. Inflammation-dependent cerebral deposition of serum amyloid a protein in a mouse model of amyloidosis. J Neurosci 22:5900–5909. https://doi.org/10.1523/JNEUROSCI.22-14-05900.2002.
152. Nadell CD, Drescher K, Foster KR. 2016. Spatial structure, cooperation, and competition in biofilms. Nat Rev Microbiol 14:589–600. https://doi.org/10.1038/nrmicro.2016.84.
153. DePas WH, Chapman MR. 2012. Microbial manipulation of the amyloid fold. Res Microbiol 163:592–606. https://doi.org/10.1016/j.resmic.2012.10.009.
154. Pistollato F, Sumalla Cano S, Elio I, Masias Vergara M, Giampieri F, Battino M. 2016. Role of gut microbiota and nutrients in amyloid formation and pathogenesis of Alzheimer disease. Nutr Rev 74:624–634. https://doi.org/10.1093/nutrit/nuw023.
155. Zhao Y, Lukiw WJ. 2015. Microbiome-generated amyloid and potential impact on amyloidogenesis in Alzheimer's disease (AD). J Nat Sci 1:e138.
156. Syed AK, Boles BR. 2014. Fold modulating function: bacterial toxins to functional amyloids. Front Microbiol 5:401. https://doi.org/10.3389/fmicb.2014.00401.
157. Shoemark DK, Allen SJ. 2015. The microbiome and disease: reviewing the links between the oral microbiome, aging, and Alzheimer's disease. J Alzheimers Dis 43:725–738. https://doi.org/10.3233/JAD-141170.
158. Hill JM, Lukiw WJ. 2015. Microbial-generated amyloids and Alzheimer's disease (AD). Front Aging Neurosci 7:9. https://doi.org/10.3389/fnagi.2015.00009.
159. Hill JM, Bhattacharjee S, Pogue AI, Lukiw WJ. 2014. The gastrointestinal tract microbiome and potential link to Alzheimer's disease. Front Neurol 5:43.
160. Sochocka M, Donskow-Łysoniewska K, Diniz BS, Kurpas D, Brzozowska E, Leszek J. 2019. The gut microbiome alterations and inflammation-driven pathogenesis of Alzheimer's disease: a critical review. Mol Neurobiol 56:1841–1851. https://doi.org/10.1007/s12035-018-1188-4.
161. Vogt NM, Kerby RL, Dill-McFarland KA, Harding SJ, Merluzzi AP, Johnson SC, Carlsson CM, Asthana S, Zetterberg H, Blennow K, Bendlin BB, Rey FE. 2017. Gut microbiome alterations in Alzheimer's disease. Sci Rep 7:13537. https://doi.org/10.1038/s41598-017-13601-y.
162. Harris SA, Harris EA. 2015. Herpes simplex virus type 1 and other pathogens are key causative factors in sporadic Alzheimer's disease. J Alzheimers Dis 48:319–353. https://doi.org/10.3233/JAD-142853.
163. Miklossy J, McGeer PL. 2016. Common mechanisms involved in Alzheimer's disease and type 2 diabetes: a key role of chronic bacterial infection and inflammation. Aging 8:575–588. https://doi.org/10.18632/aging.100921.
164. Asti A, Gioglio L. 2014. Can a bacterial endotoxin be a key factor in the kinetics of amyloid fibril formation? J Alzheimers Dis 39:169–179. https://doi.org/10.3233/JAD-131394.
165. Sharon G, Sampson TR, Geschwind DH, Mazmanian SK. 2016. The central nervous system and the gut microbiome. Cell 167:915–932. https://doi.org/10.1016/j.cell.2016.10.027.
166. Miraglia F, Colla E. 2019. Microbiome, Parkinson's disease, and molecular mimicry. Cells 8:222. https://doi.org/10.3390/cells8030222.
167. Lundmark K, Westermark GT, Olsen A, Westermark P. 2005. Protein fibrils in nature can enhance amyloid protein A amyloidosis in mice: cross-seeding as a disease mechanism. Proc Natl Acad Sci U S A 102:6098–6102. https://doi.org/10.1073/pnas.0501814102.
168. Friedland RP. 2015. Mechanisms of molecular mimicry involving the microbiota in neurodegeneration. J Alzheimers Dis 45:349–362. https://doi.org/10.3233/JAD-142841.
169. Cui D, Kawano H, Hoshii Y, Liu Y, Ishihara T. 2008. Acceleration of murine AA amyloid deposition by bovine amyloid fibrils and tissue homogenates. Amyloid 15:77–83. https://doi.org/10.1080/13506120802005833.
170. Miglio A, Moscati L, Fruganti G, Pela M, Scoccia E, Valiani A, Maresca C. 2013. Use of milk amyloid A in the diagnosis of subclinical mastitis in dairy ewes. J Dairy Res 80:496–502. https://doi.org/10.1017/S0022029913000484.
171. Villar-Pique A, Sabate R, Lopera O, Gibert J, Torne JM, Santos M, Ventura S. 2010. Amyloid-like protein inclusions in tobacco transgenic plants. PLoS One 5:e13625. https://doi.org/10.1371/journal.pone.0013625.
172. Malishev R, Abbasi R, Jelinek R, Chai L. 2018. Bacterial model membranes reshape fibrillation of a functional amyloid protein. Biochemistry 57:5230–5238. https://doi.org/10.1021/acs.biochem.8b00002.
173. Osterlund N, Kulkarni YS, Misiaszek AD, Wallin C, Kruger DM, Liao Q, Mashayekhy Rad F, Jarvet J, Strodel B, Warmlander S, Ilag LL, Kamerlin SCL, Graslund A. 2018. Amyloid-beta peptide interactions with amphiphilic surfactants: electrostatic and hydrophobic effects. ACS Chem Neurosci 9:1680–1692. https://doi.org/10.1021/acschemneuro.8b00065.

Nir Salinas received his bachelor's degree in Molecular Biochemistry from the Schulich faculty of Chemistry at the Technion–Israel Institute of Technology in 2015. Nir completed his Ph.D. in structural biology and biophysics under the supervision of Prof. Meytal Landau (2020) at the faculty of biology at the Technion. Nir is the recipient of the prestigious Clore Fellowship. His doctoral research focused on the structural, biophysical and biological studies of functional amyloids and antimicrobial peptides. Nir is now a postdoctoral researcher at the laboratory of Prof. Daniel Minor at the University of California—San Francisco.

Tatyana L. Povolotsky graduated from the University of California San Diego with a B.Sc. degree in Molecular Biology and an M.Sc. degree in 2009 under the supervision of Prof. Milton Saier, Jr. She was awarded the competitive DFG GRK 1673: Indo German Research Training Group "Functional Molecular Infection Epidemiology" fellowship from 2010 to 2013 for pursuing her doctoral degree. She earned her Ph.D. from Freie Universität Berlin under the supervision of Prof. Regine Hengge in 2014 and continued to work in the lab until 2016, elucidating the trends of c-di-GMP signaling with respect to biofilm formation and stress response in *E. coli*. She is currently a postdoctoral fellow at the Weizmann Institute of Science in the lab of Ilana Kolodkin-Gal. Tatyana studies the ability of *B. subtilis* to form community biofilms with related *Bacillus* species.

Meytal Landau received her bachelor's degree in Pharmacy from the Hebrew University of Jerusalem, Israel (2000), and her Master's in neurobiology (2002) and Ph.D. in computational biochemistry (2008) from Tel-Aviv University, Israel. In 2007, she joined the laboratory of Prof. David Eisenberg at University of California—Los Angeles as a postdoctoral researcher. In 2012, Landau opened her research lab at the faculty of Biology at the Technion–Israel Institute of Technology, where she has been an Associate Professor since 2019. Prof. Landau is also a visitor group leader with the European Molecular Biology Laboratory (EMBL), Hamburg, Germany. Her research aims are to define structure-function-fibrillation relationships and the mechanism of toxicity of protein fibrils serving as key virulence determinants in bacteria, or acting as antimicrobials in prokaryotes and eukaryotes, and which are possibly involved in systemic and neurodegenerative diseases.

Ilana Kolodkin-Gal received her bachelor's degree in Medical and Life Sciences from Tel Aviv University, Israel (2003). Ilana completed her Ph.D. *summa cum laude* in Microbiology under the supervision of Prof. Hanna Engelberg-Kulka (2009) at the faculty of Medicine in the Hebrew University of Jerusalem. Ilana is the recipient of the prestigious Katzir award. Her doctoral research focused on quorum sensing and microbial cell death. In 2009, she joined the laboratory of Prof. Richard Losick at Harvard University, Boston, MA, as a postdoctoral researcher and an EMBO and HFSP fellow. In 2013, Ilana opened her research lab at the faculty of Biochemistry at the Weizmann Institute of Science. Her research aims are to define structure-function relationships in microbial communities and their impact on beneficial (host defense) and deleterious (virulence) communal behaviors.